

A new species of the giant pill-millipede genus *Sphaerobelum* Verhoeff, 1924 from northern Thailand, with an extensive description and molecular characters (Diplopoda: Sphaerotheriida: Zephroniidae)

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Abstract

As a first step towards an inventory of the giant pill-millipedes in Thailand, a new species of the genus *Sphaerobelum* Verhoeff, 1924, *S. truncatum* **n. sp.** is described from Nan Province, northern Thailand. A determination key is presented for all five known *Sphaerobelum* species. Clear morphological differences between *S. truncatum* **n. sp.** and the other four *Sphaerobelum* species were found on the anterior telopods. For the first time in *Sphaerobelum*, the partial mitochondrial COI gene was sequenced for *S. truncatum* **n. sp.** and compared with distance, maximum parsimony and maximum likelihood methods to those of species from other giant pill-millipede genera. *Sphaerobelum truncatum* **n. sp.** was found to differ from all other analyzed giant pill-millipedes, including species of *Zephronia* Gray, 1832, by 22–30%, including numerous amino acid changes, supporting the separate status of *Sphaerobelum* among other giant pill-millipede genera. Maximum likelihood and parsimony analyses support the placement of *Sphaerobelum* in the Zephroniidae. Figures of all relevant structures of *Sphaerobelum truncatum* **n. sp.** are provided to allow the use of these characters in future descriptions of species of the family Zephroniidae.

Key words: giant pill-millipede, new species, arthropod, taxonomy, Thailand

Introduction

Millipede diversity in the world has been estimated to be 80,000 species (Hoffman 1979). However, up to now, approximately 8,000–12,000 species have been described (Sierwald & Bond 2007; Shear 2011). Among these, the order Sphaerotheriida contains currently 325 species (Wesener *et al.* 2010) and occupies a discontinuous geographical area which includes South Africa, Madagascar, the entire Oriental region, as well as New Zealand and Australia (Jeekel 1974; Hoffman 1982; Shelley 1999; Wesener & VandenSpiegel 2009). The order contains four families: Sphaerotheriidae in South Africa, Procyliosomatidae in Australia and New Zealand, Arthrosphaeridae restricted to southern India and Madagascar, and Zephroniidae (synonym Sphaeropoeidae) in Southeast Asia and the Sunda Islands, as well as an isolated genus on the Seychelles (Wesener & VandenSpiegel 2009). The family Zephroniidae is by far the most species-rich family of the order, with 140 species in 14 genera (Wesener *et al.* 2010).

However, the Zephroniidae are also in urgent need of revision; only four of its species could be included in a recent phylogenetic analysis (Wesener & VandenSpiegel 2009). Because of the unclear identity of the type species of the genus *Zephronia*, *Z. ovalis* Gray, 1832, a distinction between the three most species-rich genera, *Zephronia* Gray, 1832, *Castanotherium* Pocock, 1895 and *Sphaeropoeus* Brandt, 1833 is still problematic. On the other hand, some smaller genera are relatively well defined and were recently revised (e.g. Jeekel 2000; Mauriès 2001).

Most previous collections of the Thai millipede fauna before 2005 covered only small areas of Thailand. Thus, the species diversity of the Thai millipedes is most certainly underestimated. In 2005, Enghoff reported 105 species of Thai millipedes. However, recently, several studies in the extensive area (see Stoev *et al.* 2007; Enghoff *et al.* 2007; Golovatch *et al.* 2009, 2011a, 2011b; Pimvichai *et al.* 2009a, 2009b, 2010, 2011a, 2011b; Likhitrakarn *et al.* 2010a, 2010b, 2010c, 2011; Decker 2010) have increased the number of the Thai millipede fauna to 168 species. Most of them belong to the cylindrical and flat-backed millipedes, superorder Juliformia and order Polydesmida. The millipedes in order Sphaerotheriida occur throughout Thailand but to date only one species in this order, *Zephronia siamensis* Hirst, 1907, has been described from eastern Thailand (Sichang Island and Chanthaburi Province). As a first step to a more complete inventory of the Thai giant pill-millipede species, this paper reports a new species belonging to the genus *Sphaerobelum* Verhoeff, 1924. The new species was collected in 2010 from Nan Province, northern Thailand. It is the first record of a *Sphaerobelum* species from Thailand, located approximately 600–800 kilometers from the known distribution of other *Sphaerobelum* species (Fig. 1). A key to all known *Sphaerobelum* species is provided. The status of the genus *Sphaerobelum*, with a few of its putatively unique characters, is discussed.

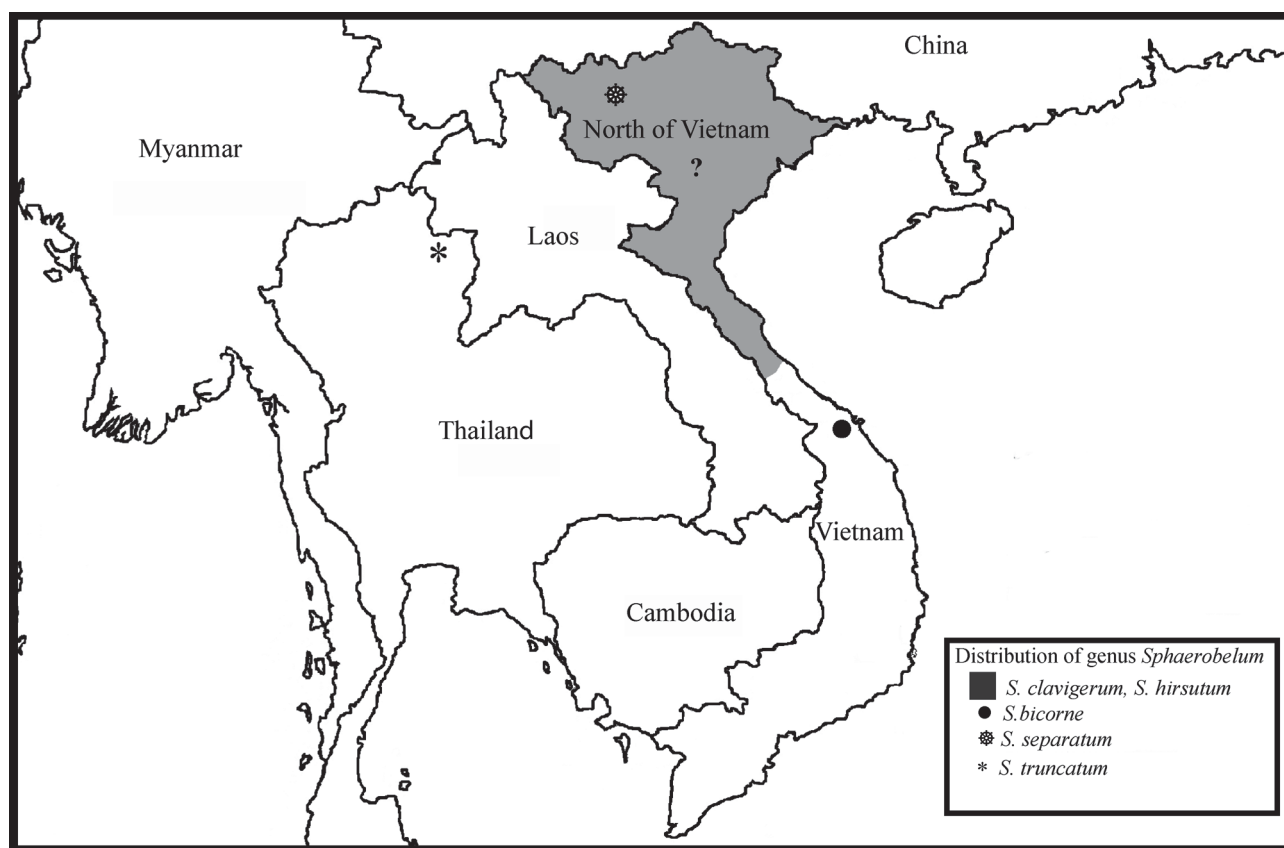


FIGURE 1. Known distribution of giant pill-millipedes of the genus *Sphaerobelum*.

Material and methods

Specimens were collected from Nan Province in August 2010 and were preserved in 70% ethanol for morphological study and DNA analysis. Some ecological and behavioral data seen during collection were recorded. All measurements are in millimeters. Terminology of morphological characters follows Van Den Spiegel *et al.* (2002) and Wesener & Sierwald (2005b). Specimens were dissected and the following body parts were removed according to the method of Wesener & Sierwald (2005a). The following characters of each sex were carefully examined:

Males: head, right antenna, gnathochilarium, left mandible, the 1st leg pair with stigmatic plate, the 2nd leg pair, the 9th leg pair with stigmatic plate, section of the 9th tergite, anterior telopod and posterior telopod.

Females: the 1st leg pair with stigmatic plate, 2nd leg pair, subanal plate.

Drawings were made with a *camera lucida* mounted on an Olympus SZH 10 research stereomicroscope. Close-up digital photos were taken with an Olympus DP20 camera mounted on an Olympus S261 stereo microscope.

SEM preparation: specimens were mounted on aluminum stubs using carbon tape, dehydrated using a vacuum type desiccator and observed under a JEOL JSM-6510A Scanning Electron Microscope.

The following type specimens were examined by TW: *Sphaerobelum clavigerum* Verhoeff, 1924 (holotype, ZMB 5747); *Sphaerobelum bicornis* Attems, 1938 (holotype, NMW 2196); *Sphaerobelum separatum* Attems, 1953 (holotype, NMW 2227). Type material of *Sphaerobelum hirsutum* Verhoeff, 1924 is unknown.

Abbreviations of repositories:

CUMZ	Museum of Zoology, Chulalongkorn University, Bangkok, Thailand
FMNH	Field Museum of Natural History, Chicago, USA
NMW	Naturhistorisches Museum Wien, Wien, Austria
ZMB	Museum für Naturkunde der Humboldt-Universität, Berlin, Germany
ZMFK	Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany

DNA extraction and sequencing. To evaluate the distinctness of *Sphaerobelum* vis-à-vis other genera of the Zephroniidae, total genomic DNA was extracted from muscle tissue laterally interconnecting the mid-body tergites from a paratype (FMNH-INS 0000 072 674) of *Sphaerobelum truncatum*, using the DNAeasy Blood & Tissue kit from Qiagen following the manufacturer's extraction protocol. The mitochondrial cytochrome oxidase subunit I (COI) gene was amplified using polymerase chain reaction (PCR) (Saiki *et al.* 1988) using the HCO/LCO primer pair (LCO-1490, HCO-2198, Folmer *et al.* 1994). PCR protocols were similar to those utilized previously (Wesener *et al.* 2010) in Sphaerotheriida. Cycle sequencing was conducted with BigDye on a Bio-rad Dyad DNA Engine, products were cleaned with ethanol on an Eppendorf centrifuge 5810R and sequenced on a Hitachi/ABI 3730 automatic DNA sequencer, using the same primer sets as for PCR. Sequencing reads were assembled with the program Seqman II (DNASTAR, Inc.), while the identity of all new sequences was confirmed with BLAST searches (Altschul *et al.* 1997). The number of base pairs obtained from *Sphaerobelum truncatum* was 674.

In order to build a framework of COI sequences for future giant pill-millipede studies in SE Asia, the COI sequence of *Sphaerobelum truncatum* was aligned by hand with COI sequences of different giant pill-millipede genera obtained during a previous study (Wesener *et al.* 2010), as well as a species of *Doratogonus* (order Spirostreptida) and *Glomeris marginata* (order Glomerida); the latter two were used to root the trees. All sequences except those of *Sphaerobelum truncatum* (Accession # JN885184) were downloaded from GenBank, for access codes see Table 1, fasta and nexus files of the dataset can be found in the Supplementary material.

TABLE 1. Specimen information and Genbank accession numbers. Species marked by an asterisk were sequenced for this analysis.

Species	Genbank Accession #
Spirostreptida, <i>Doratogonus</i> sp. GG-2003	AY288738
Glomerida, <i>Glomeris marginata</i>	FJ409909
Sphaerotheriida, unknown family, <i>Epicyliosoma</i> sp. GB	AF370841
Sphaerotheriida, Procyliosomatidae, <i>Procyliosoma leae</i>	FJ409910
Sphaerotheriida, Procyliosomatidae, <i>Procyliosoma</i> sp.	FJ409911
Sphaerotheriida, Arthrosphaeridae, <i>Arthrosphaera brandti</i>	FJ409915
Sphaerotheriida, Zephroniidae sp. Ia (<i>Zephronia</i> sp.)	FJ409912
Sphaerotheriida, Zephroniidae sp. Ib (<i>Zephronia</i> sp.)	FJ409913
Sphaerotheriida, Zephroniidae sp. II (unknown genus)	FJ409914
*Sphaerotheriida, Zephroniidae, <i>Sphaerobelum truncatum</i>	JN885184

DNA analysis. Mean pairwise distances between terminals were determined using MEGA5 (Tamura *et al.*

2011). Evolutionary analyses were conducted in MEGA5. For the maximum likelihood analysis, a model selection was undertaken with MEGA5 (Tamura *et al.* 2011) under the implemented Bayesian information criterion (BIC) which selected the Tamura-Nei model with gamma distribution (Tamura & Nei 1993) with scores: $\ln L = -3237,2$, Invariant = 0,428612, Gamma = 0,73253, $\text{Freq}_A = 0,271$, $\text{Freq}_T = 0,3463$, $\text{Freq}_C = 0,2234$, $\text{Freq}_G = 0,1593$. The bootstrap consensus tree inferred from 1,000 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein 1985). Initial tree(s) for the heuristic search were obtained automatically. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 572 positions in the final dataset.

Additionally, a maximum parsimony analysis was undertaken. The low number of taxa (10) allowed an exhaustive search of all possible tree topologies under the "ALLTREES" command to be conducted with PAUP* (v. 4.0; Swofford 2002). The dataset includes a total of 237 parsimony informative characters. A single shortest tree of length 760 was found. A parsimony bootstrap analysis (1,000 replicates) was conducted in PAUP, which resulted in a similar tree to the 'ALLTREES' search, on which maximum parsimony bootstrap values were added (Fig. 3).

The aim of this study was not a study of the phylogeny of the Zephroniidae (for which more, including nuclear, markers are needed), but a study of the distinctness of *Sphaerobelum* to support a delimitation of the genus.

Results

Genetic distance of *Sphaerobelum*: The distance matrix clearly shows that *Sphaerobelum* is distinct from other genera of Sphaerotheriida (Table 2). The uncorrected genetic distances of *Sphaerobelum* to other genera of the Zephroniidae (an undetermined genus and *Zephronia* sp. from Malaysia) are with 22.8 % (+/- 2.3%) and 23.5 % (+/- 2.2%), respectively, the lowest. Distances to other giant pill-millipedes genera vary between 26.3 % (*Epicyllosoma*) and 30 % (*Arthrosphaera*), while the genetic distances to millipedes of other orders are even higher, 31.8 % for Spirostreptida, 40.8 % for Glomerida (Table 2).

TABLE 2. Uncorrected distances of the COI gene as calculated by MEGA5.

#	Species	GenBank #	Distances in %								
			1	2	3	4	5	6	7	8	9
1	<i>Doratogonus</i> sp. GG-2003	AY288738									
2	<i>Glomeris marginata</i>	FJ409909	31.8								
3	<i>Epicyllosoma</i> sp. GB	EAF370841	30.4	36.8							
4	<i>Procyliosoma leae</i>	FJ409910	30.9	33.7	26.4						
5	<i>Procyliosoma</i> spI	FJ409911	26.0	31.8	26.4	11.9					
6	<i>Arthrosphaera brandti</i>	FJ409915	26.4	33.1	25.4	29.5	28.2				
7	Zephroniidae spIa <i>Zephronia</i> sp.	FJ409912	31.1	32.5	26.8	25.4	25.1	26.5			
8	Zephroniidae spIb <i>Zephronia</i> sp.	FJ409913	31.1	32.5	26.8	25.4	25.1	26.5	0.0		
9	Zephroniidae spII	FJ409914	29.7	36.9	25.7	25.5	25.0	27.3	22.9	22.9	
10	<i>Sphaerobelum truncatum</i>	JN885184	31.8	40.8	26.3	26.8	26.6	30.0	23.5	23.5	22.8

***Sphaerobelum* vis-à-vis other giant pill-millipede genera:** The phylogenetic analysis resulted in trees of which many branches received little statistical support. While the maximum likelihood tree (Fig. 2) as well as the maximum parsimony tree (Fig. 3) support the position of *Sphaerobelum* inside the family Zephroniidae, little else can be said about its relationship.

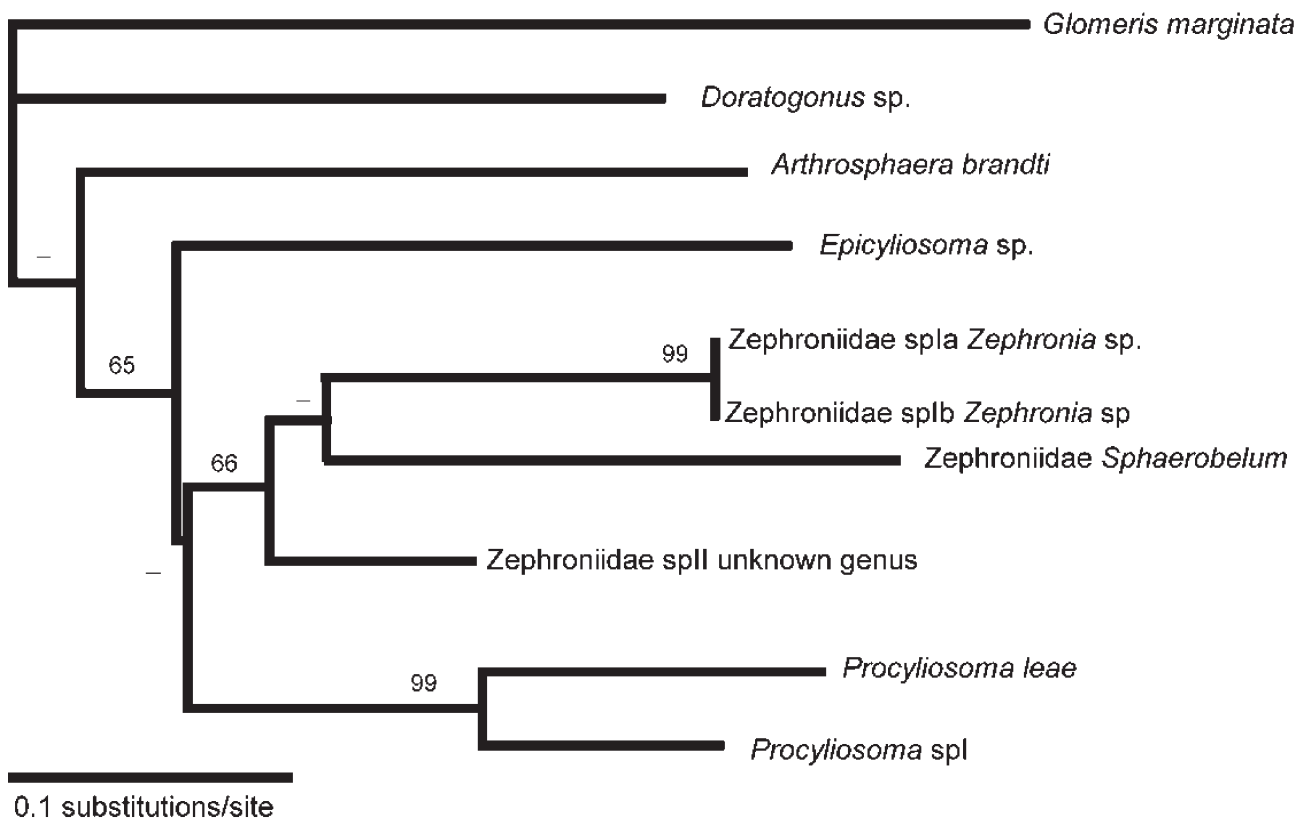


FIGURE 2. Maximum likelihood tree of the COI analysis. Bootstrap values (1,000 replicates) given above branches. Values <50% not shown (–).

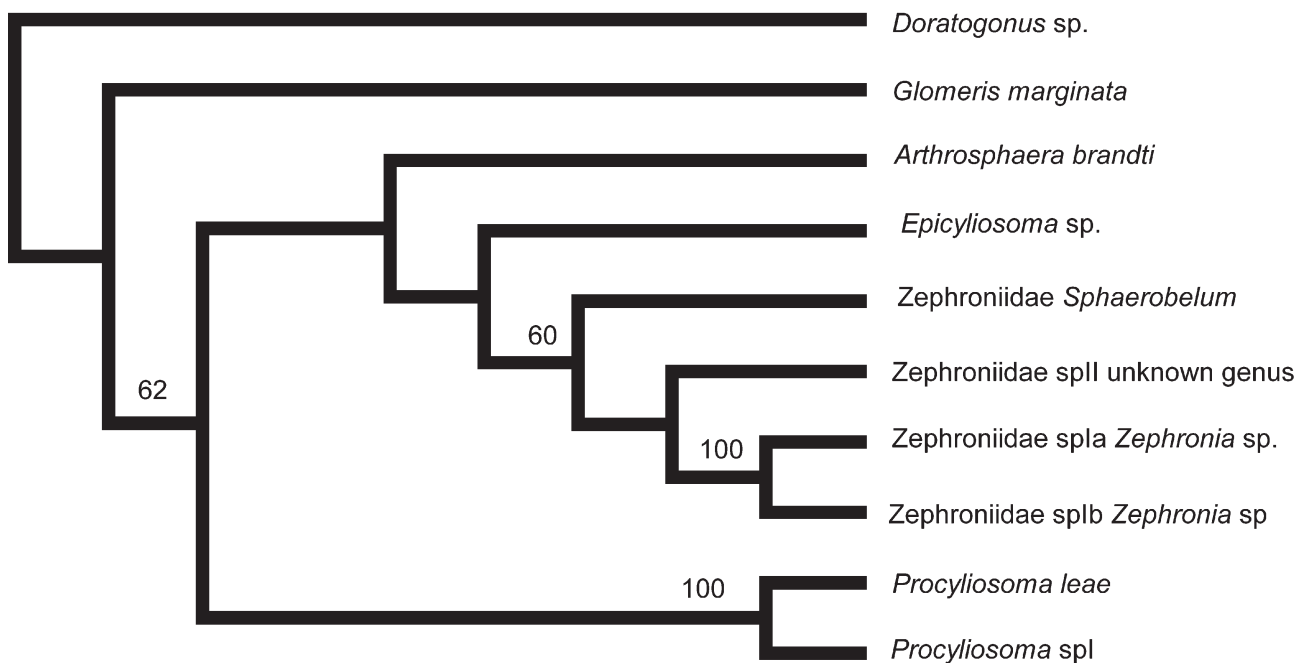


FIGURE 3. Single most-parsimonious tree obtained by the analysis of the COI dataset under the ALLTREES criterion. Length = 730. Bootstrap values >50% (1,000 replicates) given above branches.

Taxonomy

Family Zephroniidae Gray, 1843

Subfamily Zephroniinae Gray, 1843

Tribe Zephroniini Jeekel, 2001

Comment. We follow Jeekel's (2001) classification of Zephroniidae. No phylogenetic analysis of the family Zephroniidae was ever undertaken, so any subfamilial or tribal affiliations of *Sphaerobelum* would be premature.

Genus *Sphaerobelum* Verhoeff, 1924

A complete synonymy is provided by Jeekel (2001: 17)

Type species. *Sphaerobelum clavigerum* Verhoeff, 1924

Other species included. *S. hirsutum* Verhoeff, 1924; *S. bicornis* Attems, 1938; *S. separatum* Attems, 1953, *S. truncatum* n. sp.

Diagnosis. Males of *Sphaerobelum* species can be distinguished from all other giant pill-millipede genera in the world by the distally swollen process of the second joint of the posterior telopod.

Comments. A comprehensive description of the genus *Sphaerobelum* cannot be given at the moment. However, all species currently assigned to the genus display the following unique combination of characters: posterior telopods consisting of four podomeres and with a large process on the 2nd podomere which features a greatly enlarged, swollen-looking tip (Fig. 11D). The latter feature has been described for all four species included in the genus and figured for three of them (Verhoeff, 1924, fig. 36 for *S. clavigerum*; Attems, 1938, figs. 18, 19 for *S. bicornis*; Attems, 1953, fig. 27 for *S. separatum*). Special attention should be given to the minute Tömösváry organ, which is separated by a sclerotized crest from the eye field at least in some *Zephronia* species, a structure not present in *Sphaerobelum*.

Key to species of *Sphaerobelum*

1. Anterior telopod with 3 podomeres. 2
- Anterior telopod with 4 podomeres. 4
2. Tergites shiny, glabrous. *S. clavigerum* Verhoeff, 1924
- Tergites hairy. 3
3. Dirty olive-brown; head punctured and densely hairy; process of 2nd podomere of posterior telopod apically strongly swollen, spherical, wider than basis of 3rd podomere. *S. hirsutum* Verhoeff, 1924
- Black; head smooth; process of 2nd podomere of posterior telopods truncated; operculum of vulva two-horned, both horns blunt *S. bicornis* Attems, 1938
4. Podomere 3 of anterior telopod distally with lateral extension; podomere 4 of posterior telopod distally with incurved process, overlapping with process of podomere 2; marginal setae of tergite not reaching posterior margin *S. separatum* Attems, 1953
- Podomere 3 of anterior telopod cylindrical, without lateral extension; podomere 4 of posterior telopod without incurved process, but with 3 separated spines; marginal setae of tergite reaching posterior margin *S. truncatum* n. sp.

Sphaerobelum truncatum Wongthamwanich, n. sp.

Figs. 5–11

Material examined. Holotype male, **CUMZ 2010.11**, Thailand, Nan Province, Song Khwae District, Na Rai Luang Sub-district, Pang Hi Village; secondary forest; Latitude: 19.3962, Longitude: 100.6951; coll. N. Wongthamwanich; hand collecting; 22 August 2010. Paratypes: 4 males, **CUMZ 2010.12–15**, 4 females, **CUMZ 2010.16–19**, 1 male, **FMNH-INS 0000 072 674**, 1 female, **FMNH-INS 0000 072 673**, 1 male, **ZFMK Myr 001**, 1 female, **ZFMK Myr 002**, same data as holotype.

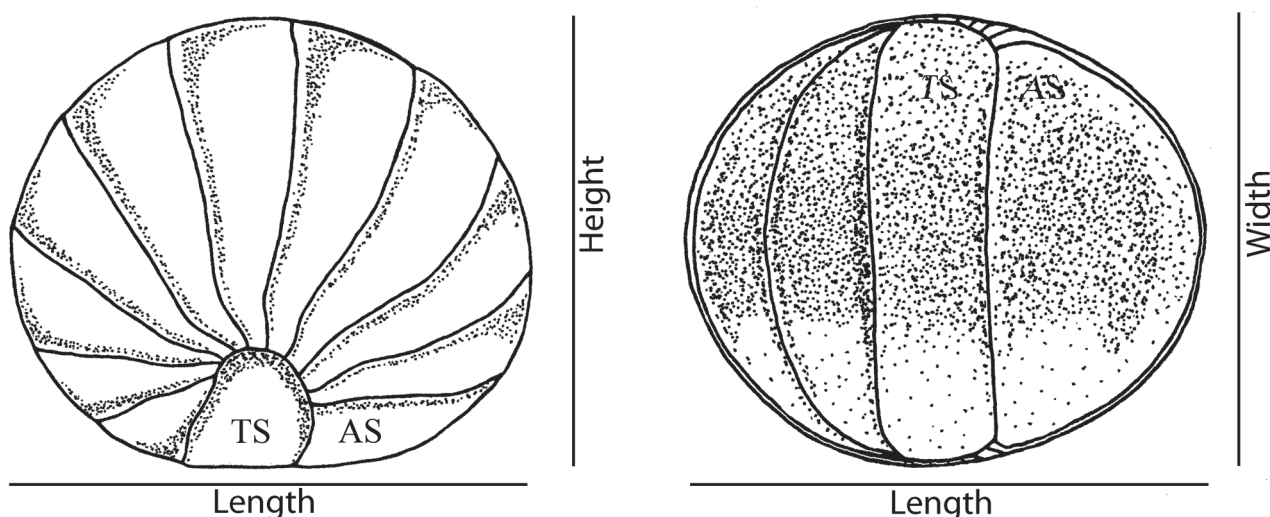


FIGURE 4. Measurements of a volvated giant pill-millipede. Abbreviations: TS = thoracic shield; AS = anal shield.

Etymology. The specific epithet is a Latin adjective, meaning terminating abruptly, and refers to the truncated state of the lateral end of the third podomere on the anterior telopods (Fig. 11C).

Diagnosis. *Sphaerobelum truncatum* differs from all other *Sphaerobelum* species in the unique combination of the following characters of the anterior telopods (Figs. 11A–C): four podomeres above syncoxite, second podomere posteriorly with a process (Figs. 11B, C) and one spine on the anterior side of the inner margin (Fig. 11C), third and fourth podomeres located posteriorly juxtaposed to process of second podomere, third podomere distally truncate, fourth podomere with three sclerotized spines all located on small thin separated sclerotized plates: one spine at apex, the other two on posterior side (Figs. 11B, C). Anterior telopods without sclerotized teeth. *S. truncatum* differs from the closely related species *S. separatum* Attems, 1953 in the presence of a well-rounded fourth podomere on the posterior telopod (Figs. 11D, E), while the tip of the posterior telopod in the latter species is prolonged into a thin process and curved towards the process of the second podomere. *S. truncatum* is the only giant pill-millipede species known to have a regularly ‘square-wavy’ margin on the endotergum (Fig. 9B).

COI Sequence (paratype, **FMNH-INS 0000 072 674**): Accession # JN885184. See supplementary material.

Description. *Measurements*: Males: length 18.6–24.0 mm, width at the seventh tergite 10.2–12.6 mm, width of thoracic shield 9.9–12.2 mm, height of thoracic shield 5.7–6.8 mm. Sizes of volvated male (for epimorphic stadia, Figs. 4A, B): length (tergite 5–tergite 11 or 12) 12.1–13.9 mm, width (tergite 7) 10.2–12.6 mm, and height (thoracic shield–tergite 8) 10.7–12.6 mm. Females: length 21.2–30.4 mm, width at the seventh tergite 11.5–15.2 mm, width of thoracic shield 11.0–14.3 mm, height of thoracic shield 6.2–8.1 mm. Sizes of volvated female: length 13.0–17.6 mm, width 11.5–15.2 mm, and height 11.5–15.4 mm.

Habitus (Fig. 5C): Overall shape elliptical, tapering slightly from sixth tergite towards head.

Coloration (Figs. 5A–C): head dark brown, collum, thoracic shield, tergite, and anal shield black. Antennae and legs light yellow.

Head: trapezoid, anterior part of the head with many long setae, posterior part densely dimpled; anterior margin of labrum with a single tooth. Eye field round, width approximately one mm, consisting of more than 60 densely-packed ocelli. No sclerotized crest/ridge between antenna socket and eye field. Tömösváry organ (Fig. 5D) between eye field and antenna socket, next to, but separated from eye field.

Antennae (Figs. 6A–C): with six visible antennomeres; basal antennomere with a few thinly scattered sclerotized nodules (Fig. 6C); distal antennomere enlarged, round and club-shaped, with 81–84 sensory cones in males, 36–42 in females. Antennomere lengths: $6 > 1 > 2 = 3 > 4 = 5$.

Mandibles (Figs. 7A, B): with a single external tooth, a three-lobed internal tooth, six rows of pectinate lamellae, number of teeth on pectinate lamellae declining from apical to proximal lamella, molar plate with one visible ridge.

Gnathochilarium (Fig. 7C): with numerous long setae on the lingual lamella. Inner palpi with numerous elongate and sharp sensory cones (Figs. 7D, E), reduced lateral palpi with a field of 6 sensory cones (Figs. 7D, F).

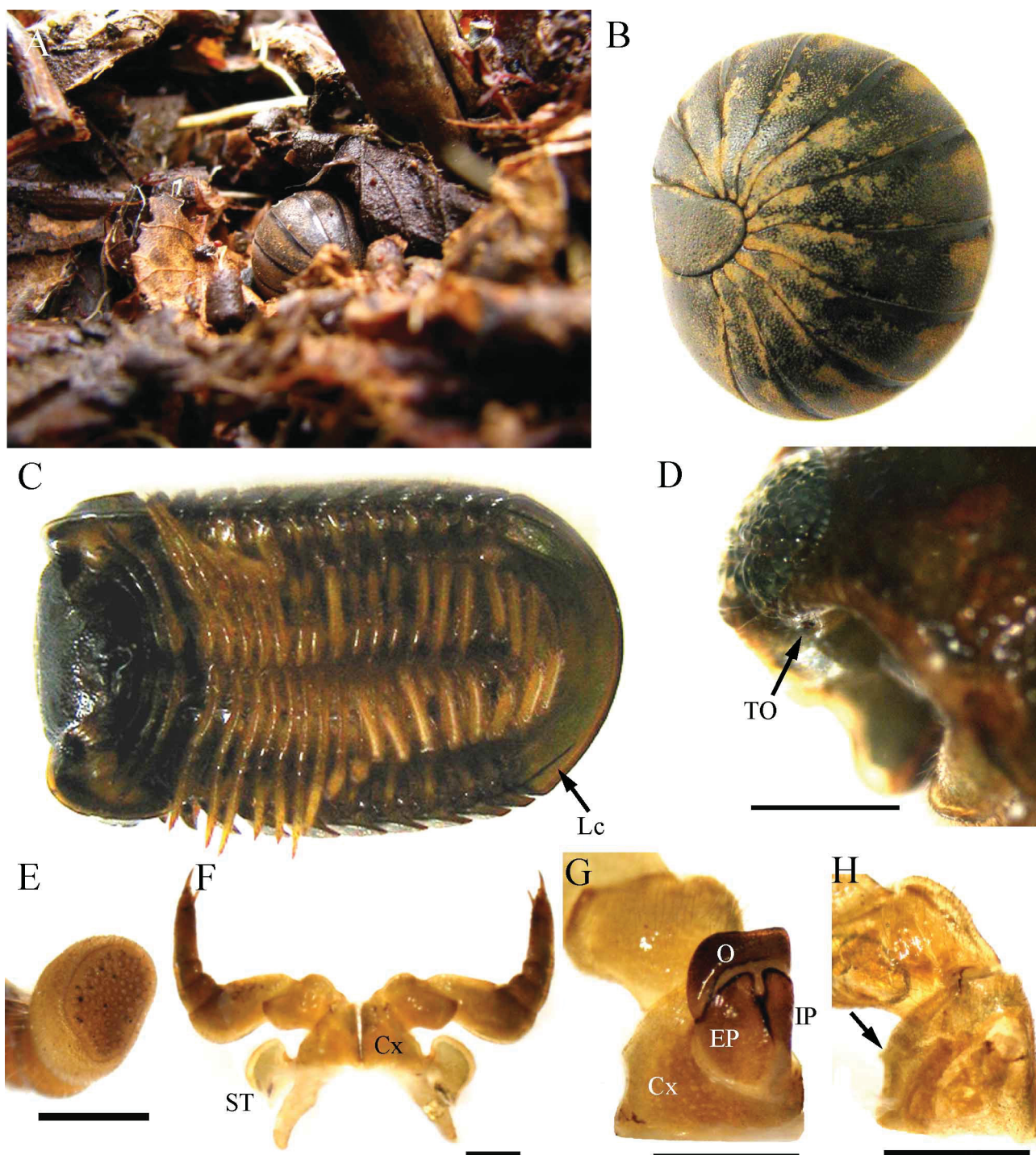


FIGURE 5. *Sphaerobelum truncatum* n. sp., **Digital photographs**, **A**: paratype in habitat; **B**: paratype, living specimen voluted; **C**: paratype, ventral view, arrow points to locking carina of anal shield; **D**: eye field and Tömösváry organ; **E**: distal right antennomere; **F**: holotype, first pair of legs with stigmatic plate; **G**: paratype, vulva of female; **H**: holotype, second coxa, arrow points to coxal lobe. Abbreviations: **Lc** = locking carina; **TO** = Tömösváry organ; **Cx** = coxa; **ST** = stigmatic plate; **O** = operculum; **EP** = external plate of vulva; **IP** = mesal plate of vulva. **A-C**: Photographs not to scale. Scale bars = 1 mm.

Collum: anterior and posterior margin with a few setae, center part sparsely setae, densely covered by dimple-like impressions.

Thoracic shield: ridge on lateral lobe absent, accompanied by a few long setae; center part of anterior margin covered sparsely with long setae.

Tergites 3–12 (Fig. 8): Anterior margins dominated by small conical spines (Fig. 8C). Two submarginal rows of round sclerotized nodules: nodules of anterior-most row large and isolated, those of second row small and close

together (Fig. 8B). Anterior fifth of tergite with smooth zone, followed by a zone of 3–5 irregular rows of long setae, and then by an area of short setae (Fig. 8B). Central four fifths of tergite dominated by short setae, nodules and slightly curved long setae inserted in large dimples (Figs. 8A, D). Posterior margins with a few thick setae (Fig. 8A).

Endotergum (Fig. 9): with a regularly ‘square-wavy’ margin (Fig. 9B), outer zone with three rows of irregular marginal setae not reaching posterior margin. A single row of small elliptical cuticular impression present next to marginal ridge. Intersegmental membrane smooth, without cones or setae (Fig. 9A).

Anal shield: densely covered with long setae; round in both sexes. Inner side with three or four striae. Ventral side with one long black locking carina (Fig. 5C, Lc).

Legs: first pair of legs without coxal lobe (Fig. 5F), first tarsi with two ventral spines and without apical spine; second pair of legs with small coxal lobes in male (Fig. 5H), second tarsi with four ventral spines and without apical spine; third leg pair with acute coxal lobes, third tarsi with five or six ventral spines and one apical spine; ninth pair of legs with seven ventral spines and one apical spine on tarsus, femur with one long ridge (Fig. 11G). Femur of ninth pair of leg 2.0 times longer than wide, tarsus 4.8 times longer than wide (Fig. 11G).

Stigmatic plate of first leg (Fig. 5F): rounded, short and weakly curved, but forming a steep angle towards the coxa.

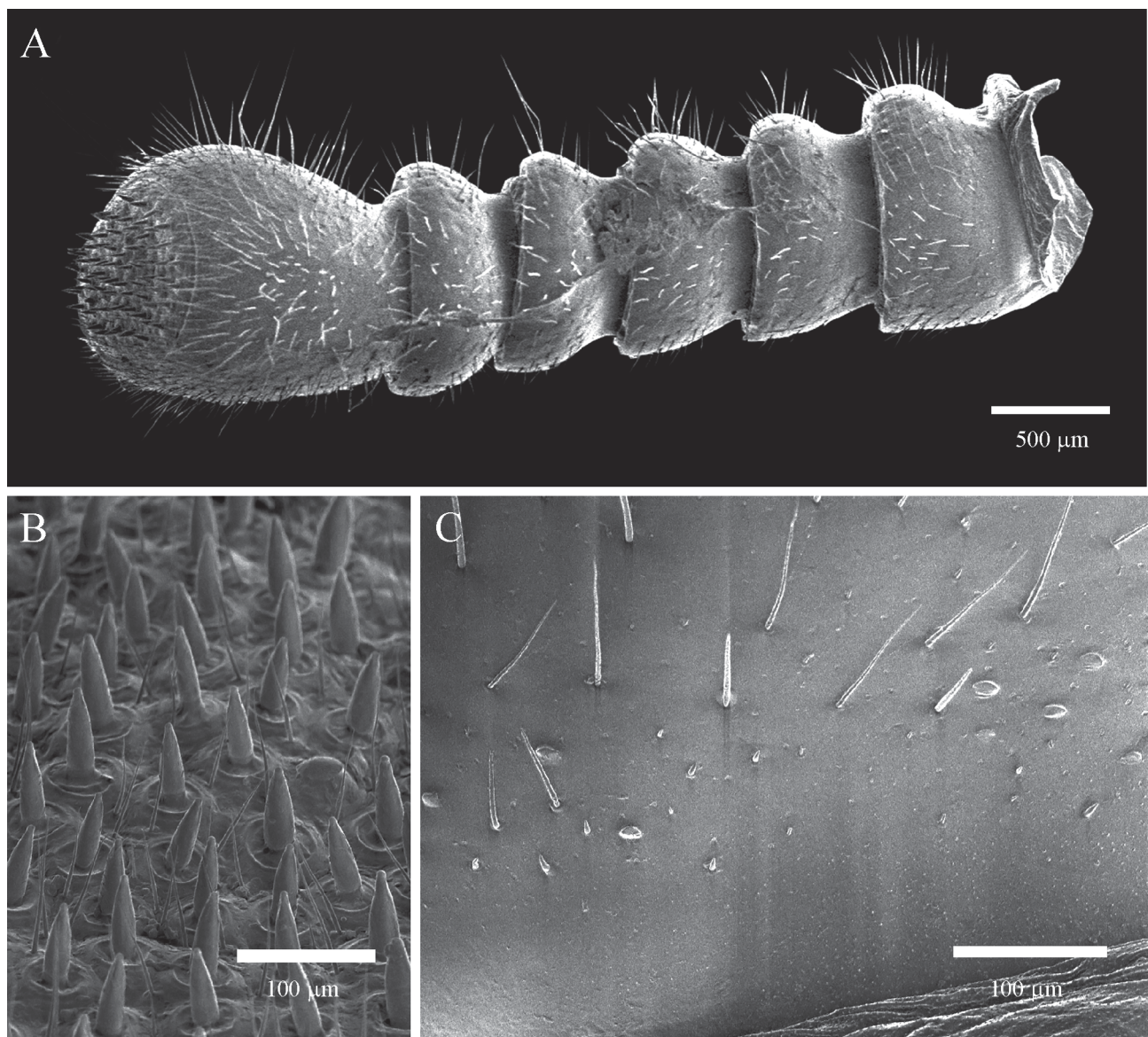


FIGURE 6. *Sphaerobelum truncatum* n. sp., holotype, SEM, **A**: right antenna, lateral view; **B**: sensory cones on distal antennomere; **C**: basal antennomere, lateral view.

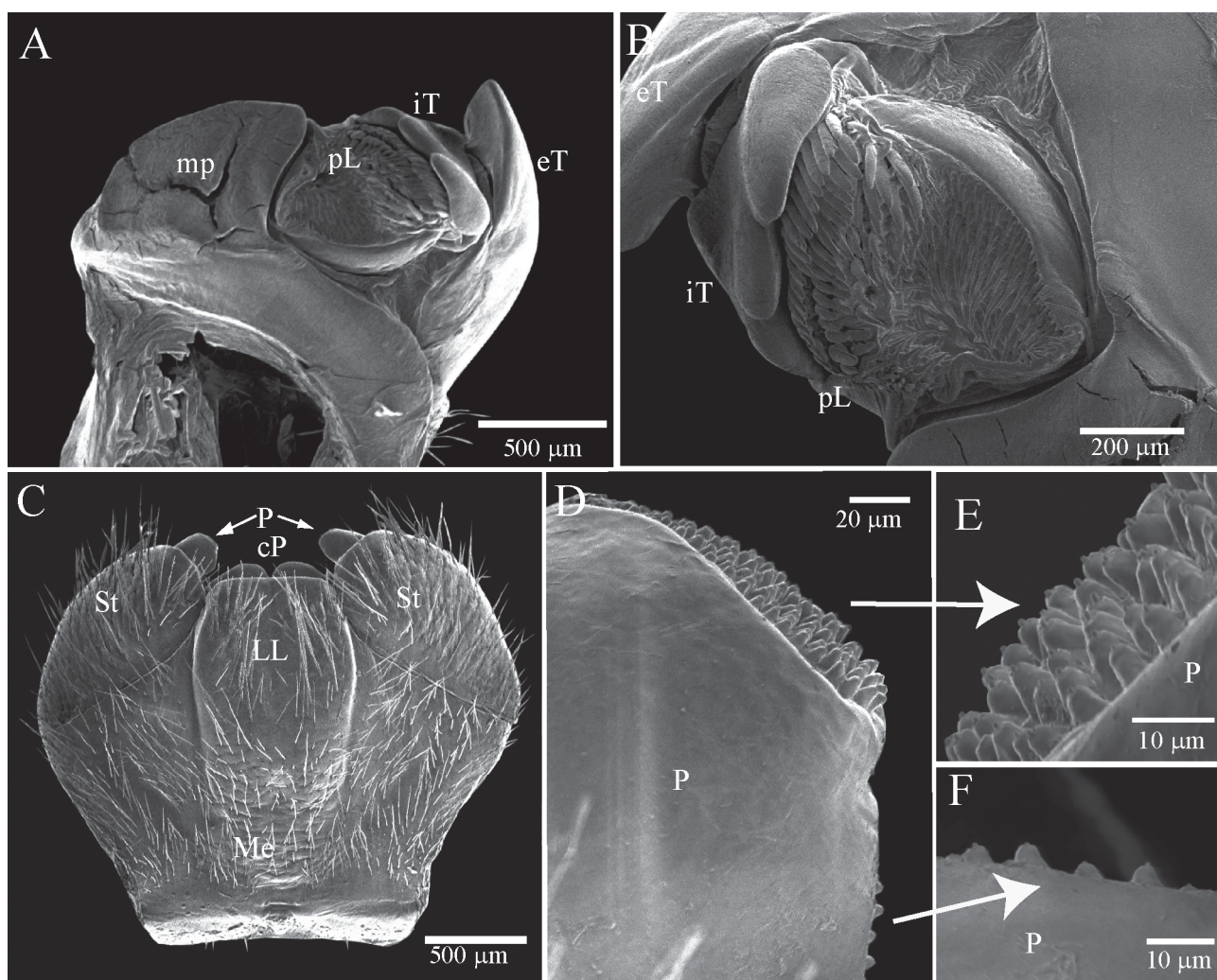


FIGURE 7. *Sphaerobelum truncatum* n. sp., holotype, SEM, **A–B**: left mandible; **C**: gnathochilarium ventral side; **D**: right palpus with sensory cone; **E**: distal sensory cones on right pulpus, **F**: sensory cones on right pulpus. Abbreviations: **mp** = molar plate; **pL** = pectinate lamella; **iT** = inner tooth; **eT** = external tooth; **P** = palpus; **cP** = central pads; **LL** = lingual lamella; **Me** = mentum; **St** = stripes.

Female vulva (Figs. 5G, 11H): consisting of two basal plates which are fused proximally and divided distally. Operculum pointed, basal part of operculum surrounding apical part of basal plates, distally protruding above coxa to basal half of prefemur. Vulva covers half (1/2) of coxa width, vulva is one-third (1/3) longer than coxa.

Subanal plate (Fig. 10A): brown, semicircular with a central shallow notch, laterally with four rib-like structures.

Male gonopore (Fig. 10B): covered by undivided sclerotized plate.

Anterior telopods (Figs. 11A–C): syncoxite with small spines, telopodite consisting of four podomeres, all podomeres covered by long setae. First podomere width equals height. Second podomere with posterior lobe-like, curved process with rounded edges; process protruding up to end of third podomere; anterior side of process with a single spine at inner margin. Third podomere distally truncate. Fourth podomere as long as third podomere, with three sclerotized spines located on small thin sclerotized fields, one spine at apex and the other two located on posterior side. Sclerotized teeth absent.

Posterior telopods (Figs. 11D, E): inner horns with sharp-edged tips, slightly curved backwards. Telopodite consisting of four podomeres. First podomere parallelogram-shaped. Second and third podomere dominated by short setae, margins with long setae. Process of second podomere weakly curved, distally glabrous, posteriorly apically enlarged and swollen (Fig. 11D), anteriorly apically concave, spatulate, with a single sclerotized spine (Fig. 11E). Moveable finger consisting of third podomere and small fourth podomere, slightly curved. Third podomere

at inner margin with one small light colored spine, without any crenulated teeth. Fourth podomere at inner margin with two small sclerotized spines located in single brown sclerotized field.

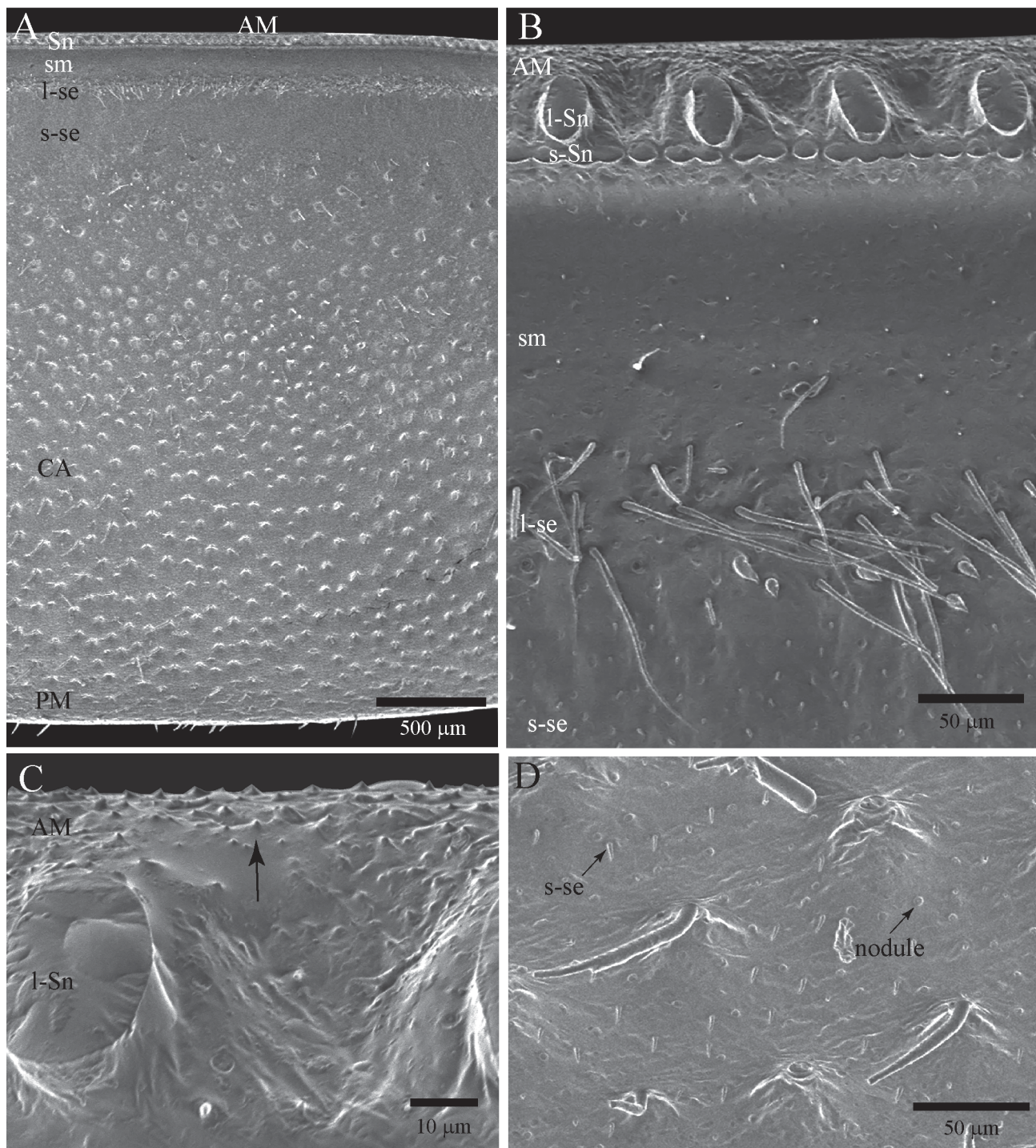


FIGURE 8. *Sphaerobelum truncatum* n. sp., holotype, SEM, **A:** tergite; **B:** anterior fifth of tergite; **C:** anterior margin of tergite, arrow points to small conical spines; **D:** setae and nodules on central area of tergite. Abbreviations: Sn = sclerotized nodules; l-se = long setae; s-se = short setae; sm = smooth area; AM= anterior margin; CA = central area; PM = posterior margin; l-Sn = large sclerotized nodules; s-Sn = small sclerotized nodules.

Intraspecific variation. Only seven males and six females of *S. truncatum* were available for analysis. However, some characters presented a clear difference between the sexes, such as the body length of females that tended to be longer than that of the males, while males have more sensory cones on the distal antennae than females.

Life history. Adult specimens were collected in late August 2010 during the rainy season. Numerous eggs were found in an adult female, with most of them aggregated into many small clusters located between the intestine and the

body wall, from the second leg pairs to posterior end of the body. The eggs as preserved in 70% ethanol were orange in color. Mature eggs were round in shape with a diameter of about 2–2.5 mm.

Distribution and ecology. The new species is only known from the type locality at 440–450 m altitude. There is a small stream running along the secondary dry evergreen forest and the forest floor is covered by laterite soil with saplings and seedlings, including a leaf litter of up to 3 cm depth. At the time of collection, the weather was a light precipitation, 92% relative humidity, 25.5–26°C and 27°C soil and air temperature respectively. Most specimens were found on a 60 degree slope and only a few specimens were found on a flat plain area. Animals were found rolled up above the forest ground close to tree bases, in shallow soil pockets and under leaf litter.

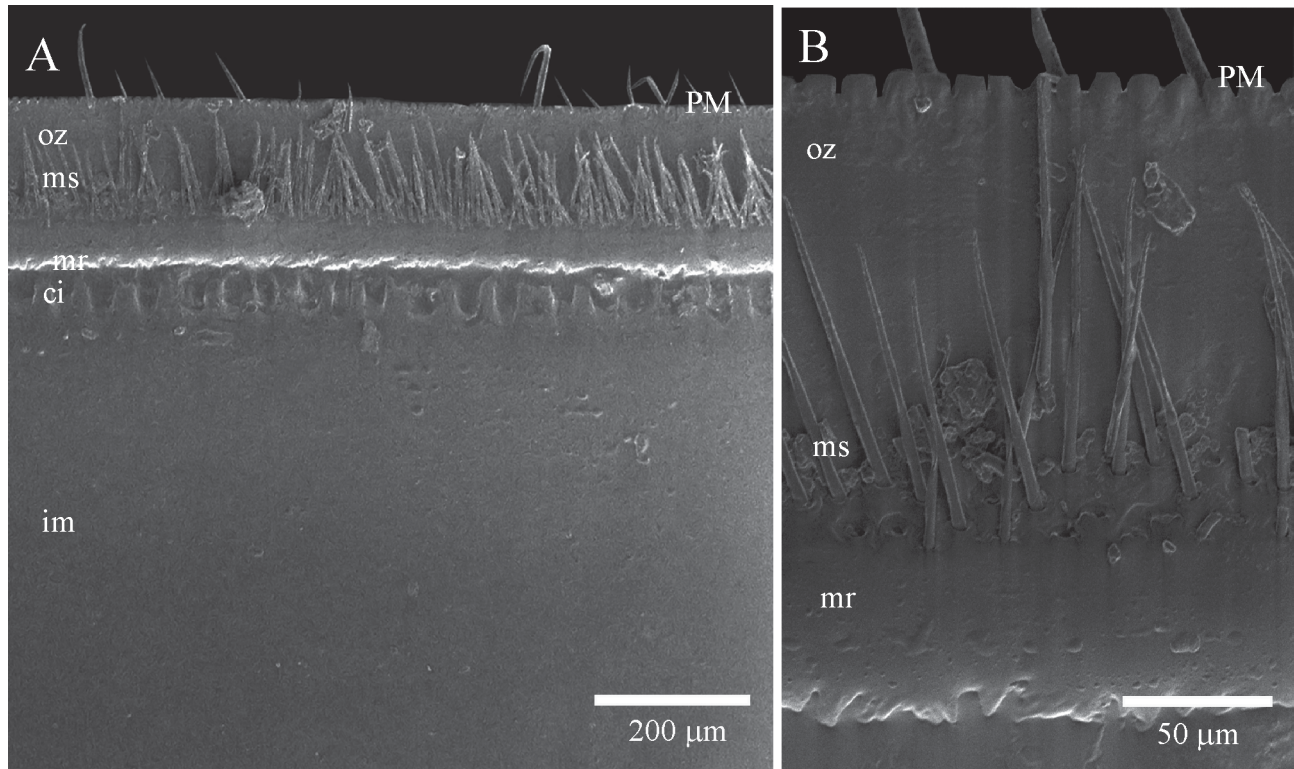


FIGURE 9. *Sphaerobelum truncatum* n. sp., holotype, endotergum, SEM, **A**: overview; **B**: posterior margin. Abbreviations: **PM** = posterior margin; **oz** = outer zone; **ms** = marginal setae; **mr** = marginal ridge; **ci** = cuticular impressions; **im** = intersegmental membrane.

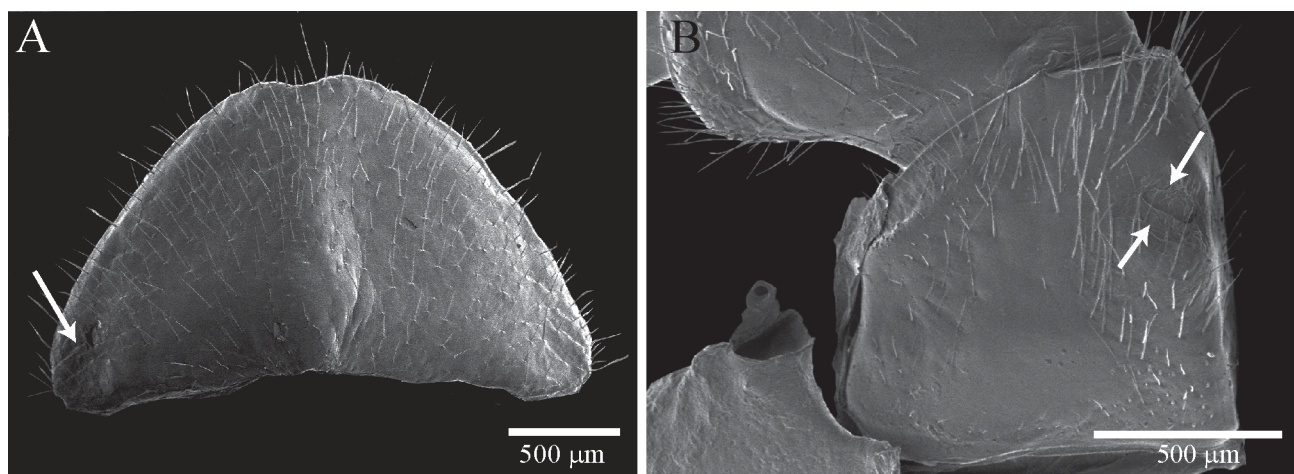


FIGURE 10. *Sphaerobelum truncatum* n. sp., SEM, **A**: female paratype, subanal plate with ribs, arrow points to ribs; **B**: holotype, male gonopore on second coxa, arrows point to gonopore.

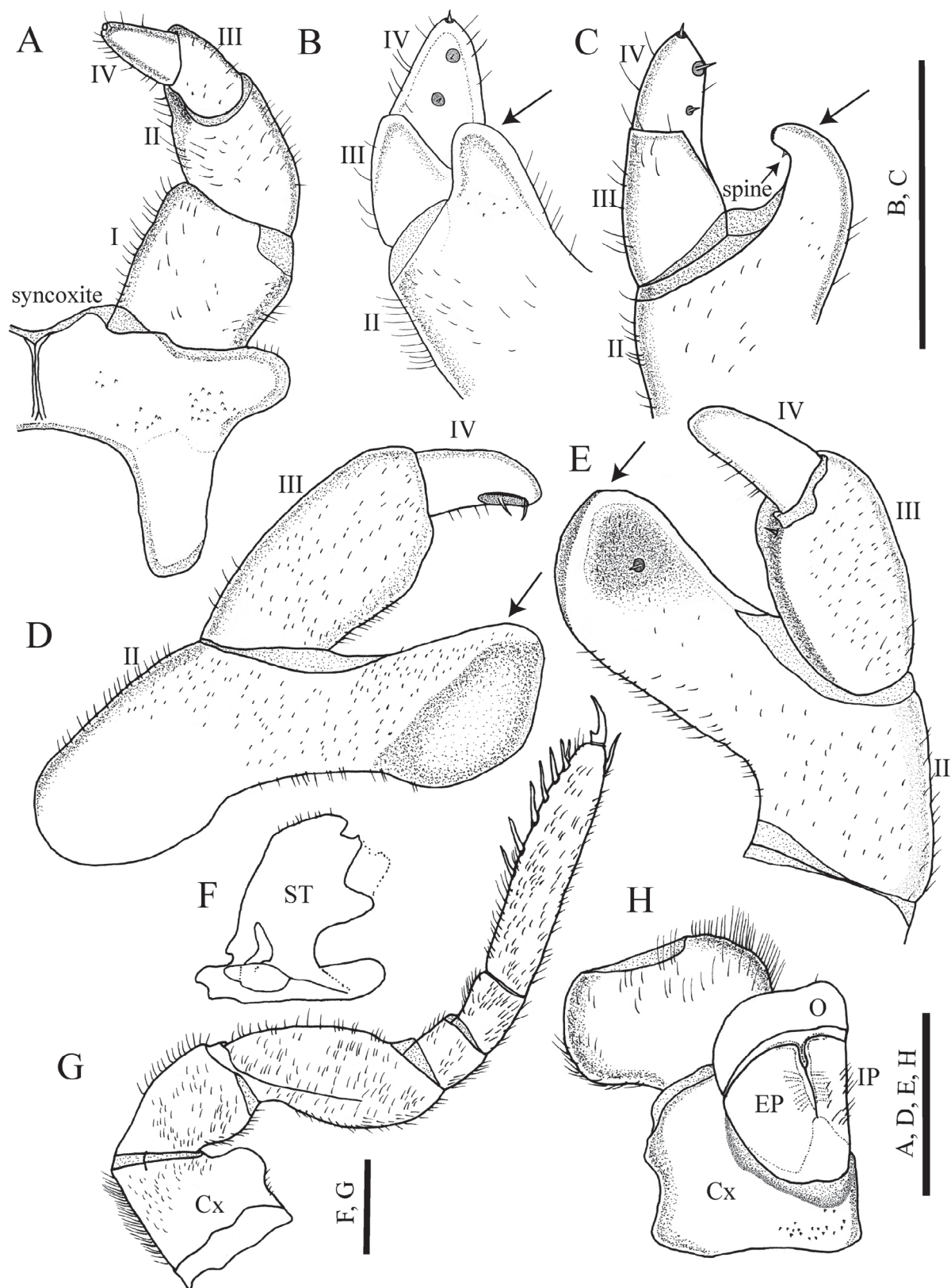


FIGURE 11. *Sphaerobelum truncatum* n. sp., A-G holotype, H female paratype, drawing, **A**: right anterior telopod, anterior view; **B**: last three podomeres of right anterior telopod, posterior view; **C**: last three podomeres of right anterior telopod, lateral view; **D**: last three podomeres of right posterior telopod, posterior view; **E**: last three podomeres of right posterior telopod, anterior view; **F**: stigmatic plate of 9th left leg; **G**: 9th left leg, posterior view; **H**: second right coxa with vulva, posterior view. Arrows point to the second podomere process. Abbreviations: **Cx** = coxa; **ST** = stigmatic plate; **O** = operculum; **EP** = external plate of vulva; **IP** = mesal plate of vulva. Roman numbers refer to number of podomere. Scale bars = 1 mm.

Behavior. *Sphaerobelum truncatum* seems to be more active than *Zephronia* sp. They unroll their body in less than 5 minutes after capturing in the hand, which has never been observed for *Zephronia* sp. *Sphaerobelum truncatum* is well camouflaged, having similar color patterns to various objects in the environment, such as dried leaves and fruit seeds. The animals can also hide during the day by digging into the soil.

Discussion

Future progress on the systematics of the Zephroniidae is currently blocked by the unclear status of the type of *Zephronia*, *Z. ovalis* Gray, 1832, a *nomen dubium* of which the first description is unsuitable for a species determination and the type specimens have been lost. Once this issue is solved, best by the selection of a neotype, the genera of the Zephroniidae should be re-defined based on their respective type species. The family Zephroniidae would be a good target for a morphological phylogenetic analysis since its members possess numerous morphological characters that could be exploited for a phylogenetic study. Probably around 100 external morphological characters could be sampled, hopefully allowing a well-resolved phylogeny. Future describers of Zephroniidae species should be aware of the fact that numerous characters are potentially useful and should be illustrated and described. While *Sphaerobelum truncatum* is clearly distinct from its congeners, a revision of the remaining *Sphaerobelum* species would allow a more precise definition of the genus.

This new species is only the second description of a sphaerotheriidan from Thailand in 100 years.

Acknowledgements

Special thanks should go to Dr. Sanong Ekgasit for permitting us to use the SEM from Sensor Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University. We thank Mr. Utis Khadpang for field assistant and Mr. Nguyen Duc Anh (Hanoi) for preparing distribution data of *Sphaerobelum* from Vietnam. This study was partly conducted at the Field Museum's Pritzker Laboratory of Molecular Systematics and Evolution headed by Dr. Kevin Feldheim. Thanks also to Dr. Verena Stagl and Mr. Edmund Schiller (Naturhistorisches Museum Wien) for kindly providing types photographs of *S. separatum* and *S. bicornis*. This research was jointly supported by PEET grants from the National Science Foundation of the United States (NSF Grants DEB 97-12438 and 05-29715) to Dr. Petra Sierwald, Dr. William A. Shear and Dr. Jason E. Bond; the Science for Locale Project under the Chulalongkorn University Centenary Academic Development plan (2008-2012); the 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund); the Thai government budget 2010, under the Research Program on Conservation and the Center of Excellence in Biodiversity, Department of Biology, Faculty of Science, Chulalongkorn University (CEB_D_23_2010) and the Post Graduate Scholarship for Research Aboard to Nattarin Wongthamwanich from Graduate School, Chulalongkorn University.

References

- Altschul, S.F., Madden, T.L., Schäffner, A.A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D.J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*, 25(17), 3389–3402.
- Attems, C. (1938) Die von Dr. C. Dawydoff in französisch Indochina gesammelten Myriopoden. *Mémoires du Muséum National d'Histoire Naturelle*, N. S. 6 (2), 187–353.
- Attems, C. (1953) Myriopoden von Indochina, Expedition von Dr. C. Dawydoff (1938-1939). *Mémoires du Muséum national d'histoire naturelle*, N. S., série A, 5 (3), 133–230.
- Decker, P. (2010) Contributions to the Myriapod fauna of Thailand - New records of millipedes and centipedes from Thailand (Myriapoda: Diplopoda, Chilopoda). *Schubartiana*, 4, 23–34.
- Enghoff, H. (2005) The millipedes of Thailand (Diplopoda). *Steenstrupia*, 29(1), 87–103.
- Enghoff, H., Sutcharit, C. & Panha, S. (2007) The shocking pink dragon millipede, *Desmoxetes purpurea*, a colourful new species from Thailand (Diplopoda: Polydesmida: Paradoxosomatidae). *Zootaxa*, 1563, 31–36.
- Felsenstein, J. (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, 39, 783–791.
- Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3 (5), 294–299.
- Golovatch, S.I., Geoffroy, J.-J., Mauriès, J.-P. & VandenSpiegel, D. (2009) Review of the millipede genus *Plusioglyphiulus* Silvestri, 1923, with descriptions of new species from Southeast Asia (Diplopoda, Spirostreptida, Cambalopsidae). *Zoosystema*, 31(1), 71–116.
- Golovatch, S.I., Geoffroy, J.-J., Mauriès, J.-P. & VandenSpiegel, D. (2011a) New species of the millipede genus *Glyphiulus*

- Gervais, 1847 from the *granulatus*-group. *Arthropoda Selecta*, 20, 65–114.
- Golovatch, S.I., Geoffroy, J.-J., Mauriès, J.-P. & VandenSpiegel, D. (2011b) The millipede genus *Plusioglyphiulus* Silvestri, 1923 in Thailand (Diplopoda, Spirostreptida, Cambalopsidae). *Zootaxa*, 2940, 1–63.
- Hirst, A.S. (1907) On four new pill-millipedes from the Malay Peninsula and Siam. *The Annals and Magazine of Natural History*, (7)20, 215–219, pl.10.
- Hoffman, R.L. (1979) *Classification of Diplopoda*. Muséum d'Histoire Naturelle, Geneva, 237 pp.
- Hoffman, R.L. (1982) Diplopoda. In: Parker, S.P. (Ed.), *Synopsis and classification of living organisms*. McGraw-Hill Book Company, New York, pp. 689–724.
- Jeekel, C.A.W. (1974) The group taxonomy and geography of the Sphaerotheriida (Diplopoda). *Symposia of the Zoological Society of London*, 32, 41–52.
- Jeekel, C.A.W. (2000) A new genus of Sphaeropoeidae from Malaysia (Diplopoda, Sphaerotheriida). *Myriapod Memoranda*, 2, 68–70.
- Jeekel, C.A.W. (2001) A bibliographic catalogue of the Asiatic Sphaerotheriida (Diplopoda). - *Myriapod Memoranda*, 3, 5–38.
- Likhitrakarn, N., Golovatch, S.I. & Panha, S. (2010a) The millipede genus *Orthomorpha* Bollman, 1893 in Thailand. 1. Revision of the *sericata*-group, with descriptions of four new species (Diplopoda, Polydesmida, Paradoxosomatidae). *Zootaxa*, 2361, 23–45.
- Likhitrakarn, N., Golovatch, S.I., Prateepasen, R. & Panha, S. (2010b) Review of the genus *Tylopus* Jeekel, 1968, with descriptions of five new species from Thailand (Diplopoda, Polydesmida, Paradoxosomatidae). *Zookeys*, 72, 23–68.
- Likhitrakarn, N., Golovatch, S.I. & Panha, S. (2010c) The millipede *Eudasyptelis setosus* (Pocock, 1894) new to the fauna of Thailand (Diplopoda: Polydesmida: Paradoxosomatidae). *Arthropoda Selecta*, 19, 215–220.
- Likhitrakarn, N., Golovatch, S.I. & Panha, S. (2011) Revision of the Southeast Asian millipede genus *Orthomorpha* Bollman, 1893, with the proposal of a new genus (Diplopoda, Polydesmida, Paradoxosomatidae). *Zookeys*, 131, 1–161.
- Mauriès, J.-P. (2001) Sur l'identité de *Zephronia hainani* Gressitt, 1941, à propos de la description d'un nouveau *Prionobelum* (Diplopoda, Sphaerotheriida, Sphaeropoeidae) de Haïnan, Chine. *Zoosystema*, 23 (1), 131–142.
- Pimvichai, P., Enghoff, H. & Panha, S. (2009a) A revision of the *Thyropygus allevatus* group. Part 1: the *T. opinatus* subgroup (Diplopoda: Spirostreptida: Harpagophoridae). *Zootaxa*, 2016, 17–50.
- Pimvichai, P., Enghoff, H. & Panha, S. (2009b) A revision of the *Thyropygus allevatus* group. Part 2: the *T. bifurcus* subgroup (Diplopoda: Spirostreptida: Harpagophoridae). *Zootaxa*, 2165, 1–15.
- Pimvichai, P., Enghoff, H. & Panha, S. (2010) The Rhynchoproctinae, a south-east Asiatic subfamily of giant millipedes: cladistic analysis, classification, four new genera and a deviating new species from north-west Thailand (Diplopoda: Spirostreptida: Harpagophoridae). *Invertebrate Systematics*, 24, 51–80.
- Pimvichai, P., Enghoff, H. & Panha, S. (20011a) A revision of the *Thyropygus allevatus* group. Part 3: the *T. induratus* subgroup (Diplopoda: Spirostreptida: Harpagophoridae). *Zootaxa*, 2941, 47–68.
- Pimvichai, P., Enghoff, H. & Panha, S. (20011b) A revision of the *Thyropygus allevatus* group. Part 4: the *T. cuisinieri* subgroup (Diplopoda: Spirostreptida: Harpagophoridae). *Zootaxa*, 2980, 37–48.
- Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B. & Erlich, H.A., (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*, 239, 487–491.
- Shear, W.A. (2011) Class Diplopoda de Blainville in Gervais, 1844. In: Zhang, Z.-Q. (Ed.) *Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness*. *Zootaxa*, 3148, 159–164.
- Shelley, R.M. (1999) Centipedes and millipedes with emphasis on North American fauna. *Kansas School Naturalist*, 45 (3), 1–15.
- Sierwald, P. & Bond, J.E. (2007) Current status of the myriapod class Diplopoda (millipedes): taxonomic diversity and phylogeny. *The Annual Review of Entomology*, 52, 401–420.
- Stoev, P., Enghoff, H., Panha, S. & Fuangarworn, M. (2007) A second species in the millipede suborder Sinocallipodidea Shear, 2000 (Diplopoda: Callipodida). *Zootaxa*, 1450, 63–68.
- Swofford, D. (2002) *PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods)*, version 4.0b. Sinauer Associates, Sunderland, MA, USA, 142 pp.
- Tamura, K. & Nei, M. (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, 10, 512–526.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution*, 28(10), 2731–2739.
- Verhoeff, K.W. (1924) Results of Dr. E. Mjöberg's Swedish scientific expedition to Australia 1910-1913. 34. Myriapoda: Diplopoda. *Arkiv för Zoologi*, 16(5), 1–142, pl.1–5.
- VandenSpiegel, D., Golovatch, S.I. & Hamer, M.L. (2002) Revision of some of the oldest species in the millipede genus *Sphaerotherium* Brandt, 1833 (Diplopoda, Sphaerotheriida, Sphaerotheriidae), with new synonymies. *African Invertebrates*, 43, 143–181.
- Wesener, T. & Sierwald, P. (2005a) New giant pill-millipede species from the littoral forest of Madagascar (Diplopoda, Sphaerotheriida, *Zoosphaerium*). *Zootaxa*, 1097, 1–60.
- Wesener, T. & Sierwald, P. (2005b) The giant pill-millipedes of Madagascar: Revision of the genus *Sphaeromimus*, with a review of the morphological Terminology (Diplopoda, Sphaerotheriida, Sphaerotheriidae). *Proceedings of the California Academy of Sciences*, 56 (29), 557–599.
- Wesener, T. & VandenSpiegel, D. (2009) A first phylogenetic analysis of giant pill-millipedes (Diplopoda: Sphaerotheriida), a new model Gondwanan taxon, with special emphasis on island gigantism. *Cladistics*, 25, 1–29.
- Wesener, T., Raupach, M.J. & Sierwald, P. (2010) The origins of the giant pill-millipedes from Madagascar (Diplopoda: Sphaerotheriida: Arthrosphaeridae). *Molecular Phylogenetics and Evolution*, 57, 1184–1193.